CHEMISTRY OF DERIVATIVES OF INOSITOL

BY

EDWIN HAROLD FLYNN

B.S., University of Nebraska, 1944

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN CHEMISTRY
IN THE GRADUATE COLLEGE OF THE
UNIVERSITY OF ILLINOIS, 1949

URBANA, ILLINOIS

1949

CHEMISTRY OF DERIVATIVES OF INOSITOL

BY

EDWIN HAROLD FLYNN

B.S., University of Nebraska, 1944

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN CHEMISTRY
IN THE GRADUATE COLLEGE OF THE
UNIVERSITY OF ILLINOIS, 1949

URBANA, ILLINOIS

1949

ACKNOWLEDGMENT

The author wishes to express his sincere appreciation to Dr. Herbert E. Carter who directed this research, for his invaluable advice, assistance, and stimulating interest.

The author is indebted to the Abbott Laboratories, Eli Lilly and Co., Parke, Davis and Co., and the Upjohn Co. for their extensive grants in support of this work.

R. UNIV. BIBLIOTHEEK LEIDEN

CHEMISTRY OF DERIVATIVES OF INOSITOL

PART I

Introduction

As a possible approach to the synthesis of aminocyclitols in general, and of 1,3-diaminotetrahydroxycyclohexane in particular, a study of the fermentation of *meso*-inositol by *Acetobacter suboxydans* was undertaken.

Discussion Of Results

The report of Dunning, *et al.* (1,2,3) that fermentation of *meso*-inositol by *Acetobacter suboxydans* produced a "diketo-inositol" prompted this investigation in the hope of obtaining the compound for use in synthesis of diaminotetrahydroxy-cyclohexanes.

The fermentation of inositol was carried out on a 3 per cent *meso*-inositol-0.1 per cent sorbitol medium for periods ranging from four to sixteen days, using *Acetobacter suboxydans* ATCC 621 and NRRLB-474 from the Northern Regional Research Laboratory. The assay procedure of Atkin, *et al.* (4) was modified for use in following inositol levels during the fermentation. It was found that nearly complete disappearance of the inositol took place in three to four days.

When a large ten-day fermentation was fractionated from water and aqueous methanol, five fractions were obtained, accounting for about 90 per cent of the inositol originally present in the medium. Since the decomposition points of fractions crystallized from aqueous methanol were unsatisfactory for purposes of comparison, the acetyl derivative, the phenylhydrazone, and the sodium salt of the oxime were used for characterization. All fractions gave yields of these derivatives which were comparable to those obtained with pure *scyllo*-inosose, the product normally attributed to *A. suboxydans*' action on *meso*-inositol (5,6,7).

The reaction of phenylhydrazine with the fermentation product gave pure *scyllo*-inosose phenylhydrazone if the reaction time was limited to one hour. With longer reaction periods it was found that a phenylosazone could also be isolated. It was evident that the phenylosazone did not originate from a diketone in the fermentation product when it was found to be formed by treating pure *scyllo*-inosose phenylhydrazone with phenylhydrazine.

The effect of incubation time on the composition of the fermentation product was studied. A very high yield of crude *scyllo*-inosose was obtained from four day fermentations. Longer periods gave slightly lower yields but did not influence the composition materially.

A quantitative determination of oxygen uptake during fermentation was also made, using incubation flasks fitted with manometers. The results showed an uptake of 0.5 mole of oxygen per mole of inositol.

Summary

The oxidation of *meso*-inositol by several strains of *Acetobacter suboxydans* has been investigated. The most active strains were found to oxidize *meso*-inositol completely in three to four days, the oxygen consumed during active fermentation approximating one atom per mole of *meso*-inositol.

Large scale fermentations were carried out and the fermentation product was fractionated. Each fraction was characterized by the preparation of the phenylhydrazone, acetate, and oxime. In the reaction with phenylhydrazine, a phenylhydrazone formed readily and nearly quantitatively, but on standing with excess reagent, a phenylosazone formed. The pure phenylhydrazone formed a phenylosazone on treatment with phenylhydrazine.

Contrary to the reports of Dunning, *et al.* (1,2,3) that a "diketoinositol" is produced in the fermentation, it has been shown conclusively that the predominant product was *scyllo*-

inosose. No evidence was obtained for the presence of a diketone in the fermentation product.

BIBLIOGRAPHY

1. Dunning, J. W., *et al.*, Science, 87, 72 (1938).
2. Dunning, J. W., Iowa State Coll. J. Sci., 14, 24 (1939).
3. Dunning, J. W., Fulmer, E. I., and Underkofler, L. A., Iowa State Coll. J. Sci., 15, 39 (1940).
4. Atkin, L., Schultz, A. S., Williams, W. L., and Frey, C. N., Ind. and Eng. Chem., Anal. Ed., 15, 141 (1943).
5. Kluyver, A. J., and Boezaarde, A. G. J., Rec. trav. chim., 58, 956 (1939).
6. Posternak, T., Helv. Chim. Acta, 24, 1045 (1941).
7. Posternak, T., Helv. Chim. Acta, 25, 746 (1942).

PART II

Introduction

The structures of certain halogen-substituted polyhydroxycyclohexanes have been investigated as an approach to the synthesis of aminodesoxyinositols.

Discussion Of Results

When *meso*-inositol was treated with acetyl bromide at elevated temperatures, a mixture of monobromopentaacetoxycyclohexane and two dibromotetraacetoxycyclohexanes together with partially acetylated derivatives, was formed (2). The main components were separated from the mixture by use of the acetyl derivatives and the free compounds. The preponderant product was β-dibromotetrahydroxycyclohexane, obtained in 58 per cent yield.

The β-dibromotetrahydroxycyclohexane was shown to be

the 1,3-isomer by oxidation with sodium metaperiodate. Two moles of periodate per mole of compound were consumed in the reaction. Titration of the reaction mixture with alkali showed the formation of 0.86 mole of acid per mole of compound present. The main reaction product, on oxidation with nitric acid, gave α,α′-dibromo-β-hydroxyglutaric acid. This acid, when treated with primary amines, gave the corresponding amides of two trihydroxyglutaric acids, i.e., when benzylamine was used two dibenzylamides were formed. When D-ribose was oxidized with nitric acid (3) and the product treated with benzylamine, the same two dibenzylamides were isolated from the reaction mixture.

Oxidation of trihydroxyglutaric acid dibenzylamide with periodate gave more than one mole-equivalent of glyoxylic acid benzylamide. In addition, an acid was formed in the reaction. These products are to be expected from trihydroxyglutaric acid dibenzylamide.

Periodate oxidation studies on α-dibromotetrahydroxycyclohexane suggested that this compound was the 1,2-isomer. However, the oxidation proceeded abnormally, so that conclusive evidence must await the isolation and characterization of degradation products.

When α-dibromotetrahydroxycyclohexane and β-dibromotetrahydroxycyclohexane were treated with 0.1N sodium hydroxide, in each case two moles of base per mole of compound were consumed in less than one hour.

Amination of β-dibromotetrahydroxycyclohexane with aqueous ammonia gave an amorphous product. Acetylation, followed by chromatography on alumina resulted in the isolation of a diacetamidotetraacetoxycyclohexane. This was partially deacetylated with methanolic ammonia to form a diacetamidotetrahydroxycyclohexane. The oxidation of this compound with periodate proceeded abnormally as evidenced by the fact that more than three moles per mole of compound was consumed. The compound was not identical with streptamine (1).

Summary

Reaction of *meso*-inositol yielded a monobromopentaacetoxycyclohexane and two dibromotetraacetoxycyclohexanes. These substances were separated by fractional crystallization procedures.

β-Dibromotetrahydroxycyclohexane was shown to be the 1,3-dibromo isomer.

Periodate oxidation of α-dibromotetrahydroxycyclohexane indicated it to be the 1,2-dibromo isomer. However, the oxidation proceeded abnormally and no final concluson could be drawn.

The reactions and properties of α,α'-dibromo-β-hydroxyglutaric acid were also investigated.

Amination of β-dibromotetrahydroxycyclohexane led to the formation of a diaminotetrahydroxycyclohexane. It is not identical with streptamine.

BIBLIOGRAPHY

1. Carter, H. E., *et al.*, Science, **103**, 540 (1946).
2. Griffin, E. G., and Nelson, J. M., J. Am. Chem. Soc., 37, 1152 (1915).
3. Levene, P. A., and Jacobs, W. A., Ber. 42, 3247 (1909).

VITA

The author was born in Dunlap, Iowa on August 16, 1920. He attended the public schools in Omaha, Nebraska, and two years after graduation from South High School, Omaha, he entered the University of Nebraska in September, 1940. He received a Bachelor of Science degree with distinction from that institution in June, 1944. He was employed by Merck and Co. as a research chemist until July, 1946, when he left their employ to enter the Graduate College of the University of Illinois. Since enrolling in the University of Illinois in September, 1946 he has held the position of Special Research Fellow.